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A microchip electrophoresis-mass spectrometric platform for fast separation and identification of enantiomers employing the partial filling technique

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ABSTRACT

A microchip electrophoresis-mass spectrometric (MCE-MS) method was developed for fast chiral analysis. The proposed MCE-MS platform deployed a glass/PDMS hybrid microchip with an easy-to-fabricate monolithic nanoelectrospray emitter. Enantiomeric MCE separation was achieved by means of the partial filling technique. A novel chip design with an arm channel connecting to the middle of the MCE separation channel for delivering the chiral selector was tested and proven valid. Enantiomeric separation of 3.4-dihydroxyphenylalanine (DOPA), glutamic acid (Glu), and serine (Ser), the selected test compounds, were achieved within 130 s with resolution values (R_s) of 2.4, 1.1, and 1.0, respectively. The proposed chiral MCE-MS assay was sensitive and had detection limits of 43 nM for L-DOPA and 47 nM for D-DOPA. The analytical platform was well suited for studies of stereochemical preference in living cells because it integrated cell culture, sample injection, chiral separation, and MS detection into a single platform. Metabolism of DOPA in human SH-SY5Y neuronal cells was studied as a model system. On-chip incubation of SH-SY5Y cells with racemic DOPA was carried out, and the incubation solution was injected and in-line assayed at time intervals. It was found that L-DOPA concentration decreased gradually as incubation time increased while the concentration of coexisting D-DOPA remained constant. The results firmly indicated that SH-SY5Y cells metabolized L-DOPA effectively while left D-DOPA intact.

1. Introduction

Many important molecules in nature are chiral. These include proteins and their constituent amino acids which control most processes within biological systems. Chirality is, therefore, a property inherent to all biological systems. A large number of biochemical processes exhibit stereospecificity and involve chiral endogenous compounds and metabolites [1–4]. Recent studies have shown that invariant left–right (LR) patterning or chirality is critical for embryonic development. The loss or reversal of LR asymmetry is often associated with malformations and disease [5]. In many cases, drug metabolism with stereochemical or prochiral selectivity may also contribute to the toxicity or adverse effects of the drug therapy. One such example is 3.4-dihydroxyphenylalanine (DOPA). L-DOPA is a popular therapeutic drug to treat Parkinson's disease [6–8]. However, it is antipode, D-DOPA, is not only inactive, but also toxic [9,10]. Therefore, study of chirality in biological systems receives an increasing research attention, which has promoted method development for chiral analysis.

Analytical methods based on various instrumental techniques, including biosensors, high-performance liquid chromatography (HPLC), and capillary electrophoresis have been developed for chiral analysis [11–14]. HPLC enantiomeric separations with mass spectrometric detection (HPLC-MS) are the methods of choice for a wide range of biological applications [15–17]. They offer highly repeatable chiral separation and high sensitivity, detection versatility, and chemical specificity of MS detection. Chiral separations based on capillary electrophoresis (CE) are normally fast [18,19]. Chiral CE coupled with mass spectrometry (CE-MS) has been reported for analysis of enantiomers [20–22]. Microchip electrophoresis (MCE), which can be regarded as a miniaturized version of classical CE performed on microchips, has been proven to be a speedy and highly efficient separation technique with many attractive microfluidic features such as precise control of flows, automation, and volumetric reduction of samples, reagents and waste. It has been applied in various biochemical and chemical applications since introduced by Manz et al. [23–29]. Microchip electrophoresis with mass spectrometric detection (MCE-MS) has

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been intensively studied due to its great potentials in bioassays. Recent reviews are available [30,31]. Although chiral MCE separations have been developed [32], few analytical methods based on chiral MCE with MS detection have not been reported so far [33].

The aim of this study was to develop a chiral MCE-MS method for fast separation and identification of enantiomers of biomedical interest. A new microchip design with an arm channel connecting to the middle of the MCE separation channel for delivering chiral selector was tested for performing partial filling chiral electrophoretic separations [34,35]. To avoid any potential contamination from non-volatile chiral selector, negatively charged sulfated β-cyclodextrin (sulfated β-CD) was tested as the chiral selector because it migrated away from the MS detector [36,37]. The proposed chiral MCE-MS analytical platform was evaluated by separating DOPA, glutamic acid (Glu), and serine (Ser) enantiomers as model analytes. It was then utilized to analyze on-chip incubation solutions of racemic DOPA with human SH-SY5Y neuronal cells to explore the stereochemical aspects of DOPA metabolism in the cells.

2. Experimental

2.1. Materials

Poly(dimethyl siloxane) (PDMS) prepolymer and the curing agent were purchased from Dow Corning (Sylgard 184 Kit, Midland, MI). Fused silica capillaries (254 μm ID, 360 μm OD) were obtained from Polymicro Technologies(Tucson, AZ). Glass slides were obtained from Silicon Valley Microelectronics (Santa Clara, CA). Hexamethyldisilazane (HMDS) was from Ultra Pure Solutions (Castroville, CA). Enantiomers of DOPA, Glu, and Ser were purchased from Sigma–Aldrich Chemical (St. Louis, MO). Milli-Q water was used throughout the work. All solutions were filtered through a nylon 0.22 μm syringe filter before use.

2.2. Microchip fabrication

The chip design is shown in Fig. 1. The microchip was composed of a glass substrate bearing the channel features and a PDMS cover. A corner of the microchip where the PDMS cover and the glass substrate were tapered into thin layers (<200 μm in thickness combined) served as the nanoESI emitter. The procedure we described previously [38] was used with modifications to create the glass substrate. Briefly, the design on a photomask with microchannels was transferred onto the glass substrate by means of UV exposure. A corner of the glass substrate was then beveled by polishing it on a sanding paper before channels (60 μm wide and 20 μm deep) were etched into the substrate in a well-stirred bath containing an etching solution of $HF:HNO_3:H_2O$ (10:20:70). The multilayer soft lithography technique [39–41] was used to fabricate the PDMS cover. A PDMS monopolymer solution prepared by mixing the PDMS prepolymer and curing agent at a 10:1 ratio was applied onto a silicon wafer that was coated with HMDS and spun

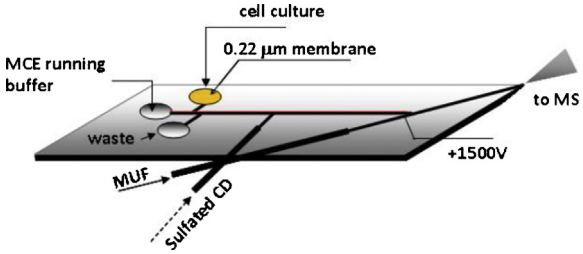

Fig. 1. Microchip design used in the proposed MCE-MS platform (channels were 60 μm wide × 20 μm deep).

at 2000 rpm for 50 s to obtain ~100 μm thick PDMS film. A platinum electrode was embedded at its location as shown in Fig. 1A to make an electric contact. After 1 h curing at 50 °C, a cofferdam was placed on top of the first layer of the PDMS cover, and filled with PDMS pre-polymer mixture, but leaving a ~5 mm long section on the cover's edge uncovered. After 3 h curing, the PDMS sheet was removed from the silicon wafer to yield a PDMS cover (~2 mm in thickness) with a ~100 μm thick section at the corner to serve as the upper layer of the nanoESI emitter. Access holes of 3-mm in diameter were drilled on the PDMS cover at channel terminals, forming the reservoirs. The microchip was made by bonding the glass substrate and the PDMS cover together through heating by means of an air plasma cleaner (10.5 W and 500 mTorr, Harrick Plasma, Ithaca, NY). A piece of 0.22 μm membrane was placed at the bottom of the sample reservoir and fixed with PDMS prepolymer solution.

2.3. MCE-nanoESI–MS system

The system consisted of an ion trap mass spectrometer (LCQ Deca, ThermoFinnigan, San Jose, CA), a microchip prepared above, a multichannel high voltage power supply, and two syringe pumps. One syringe pump was used for delivering the sulfated β-CD-containing buffer solution, and the other for MUF delivery. The microchip was fixed on a XYZ-translational stage and so positioned that the nanoESI emitter tip was about ~1.0 mm away from the MS orifice. Xcalibur software (ThermoFinnigan) was used to control the mass spectrometer and process MS data. House-written software was used for controlling the potentials applied to the microchip for MCE and nanoESI operations. MS detection conditions were optimized in positive mode and selected as follows: ion source voltage, 0 V; relative collision energy of 25% was used for MS/MS experiments with an isolation width of 1.0 u and the activation time was set at 30 ms.

2.4. Chiral MCE-MS analysis

The two syringes were filled with the respective solutions and then connected to the respective capillaries assembled into the microchip (as shown in Fig. 1). MCE running buffer was added to the buffer reservoir on the chip. The MCE channel was filled with the buffer by applying vacuum at the tip of the nano ESI emitter. MUF pump was tuned on to generate a MUF. The sulfated β-CD solution (about 35 nL) was infused to partially fill the MCE separation channel by turning on and off the syringe pump set at 250 nL/min. To inject a sample, potentials of 450 V, 500 V, 0 V, and 1000 V were applied at sample reservoir, running buffer reservoir, waste reservoir, and Pt wire (as labeled in Fig. 1) for 15 s, respectively. After sample injection, the potentials were changed to 3850 V, float, float, and 1500 V, respectively to start the MCE-MS assay. At the same time, MS data acquisition was started. When completed, the potentials were removed. MUF delivering syringe pump remained on for some time to clean the nanoESI emitter. MCE running buffer was a mixture of 15 mM ammonium acetate/acetic acid buffer (pH 5.5) and methanol (1:1). Make-up fluid was the MCE running buffer solution flowing at 100 nL/min. Sulfated β-CD solution was prepared by dissolving sulfated β-CD in the MCE running buffer at a concentration of 15 mM.

2.5. On-chip incubation of racemic DOPA with SH-SY5Y cells

The human neuronal cell line (SH-SY5Y) was purchased from The American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 100 units/mL penicillin, 10 mg/mL streptomycin, and 10% fetal bovine serum. Cells were routinely sub-cultured every 3 days. All experiments involving cell culture were conducted when cells